

Site-Specific Recombinase Strategy to create iPS Cells Efficiently with Plasmid DNA.

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Public Summary:

A breakthrough in the stem cell field occurred in 2006 when it was shown that adult cells could be turned into stem cells that were similar to embryonic stem cells. This process, known as "reprogramming" could be achieved by introducing four genes into the adult cells, turning them into what is known as induced pluripotent stem (iPS) cells. These cells have potential use as therapeutics. However, the method to make the cells involved using viruses to carry the new genes into the adult cells. The result is cells that have the virus inserted in many places, which creates mutations that can cause cancer. To make iPS cells in a safer manner, we developed new methods, described in this publication. We show here that we can turn mouse skin or fat cells into cells that are similar to embryonic stem cells. We do this without using any viruses, so the method is safer. We place the new genes into the chromosomes of the recipient cell by using a special enzyme that recognizes a DNA sequence in the cells and pastes one copy of the new genes there. We ensure that this location is safe by determining its DNA sequence and making sure that no genes are disturbed by the insertion. The new genes reprogram the cell into an iPS cell. After the reprogramming, we use another enzyme to precisely delete the reprogramming genes. The reprogramming genes are no longer needed, and they have the potential to cause cancer, so it is a good idea to remove them once reprogramming is complete. We show that our iPS cells are completely reprogrammed into stem cells by demonstrating that they express genes that are typical of embryonic stem cells, have the ability to change into a wide variety of cell types, and even have the ability to contribute tissues to a mouse when injected into a mouse embryo. Therefore, we have shown here that our new method is effective for making iPS cells, without using viruses. We expect that this new method will be helpful to many scientists who would like to use iPS cells as part of a clinical strategy.

Scientific Abstract:

Induced pluripotent stem cells (iPSC) have revolutionized the stem cell field. iPSC are most often produced by using retroviruses. However, the resulting cells may be ill-suited for clinical applications. Many alternative strategies to make iPSC have been developed, but the non-integrating strategies tend to be inefficient, while the integrating strategies involve random integration. Here we report a facile strategy to create murine iPSC that utilizes plasmid DNA and single transfection with sequence-specific recombinases. PhiC31 integrase was used to insert the reprogramming cassette into the genome, producing iPSC. Cre recombinase was then employed for excision of the reprogramming genes. The iPSC were demonstrated to be pluripotent by in vitro and in vivo criteria, both before and after excision of the reprogramming cassette. This strategy is comparable to retroviral approaches in efficiency, but is non-hazardous for the user, simple to perform, and results in non-random integration of a reprogramming cassette that can be readily deleted. We demonstrated the efficiency of this reprogramming and excision strategy in two accessible cell types, fibroblasts and adipose stem cells. This simple strategy produces pluripotent stem cells that have the potential to be used in a clinical setting.

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